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Hemochromatosis Gene Mutations in Chronic Hepatitis C Patients With and Without Liver Siderosis

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Chronic hepatitis C is often associated with liver iron overload, which may affect the long-term prognosis and the response to antiviral treatment. The occurrence of hemochromatosis (HFE) mutations were studied to determine whether may contribute to the liver iron overload of chronic hepatitis C patients. The prevalence of two HFE mutations (C282Y and H63D) in 120 chronic hepatitis C patients was determined and the findings were correlated with clinical, histological and virological features. Hepatic iron was determined semiquantitatively by a histochemical hepatic iron index, defined as the ratio of a histochemical staining score to the patient's age, after correction for heterogeneous lobular iron distribution. Serum hepatitis C virus (HCV) RNA was measured by bDNA assay and typed by restriction fragment length polymorphism. Liver HCV RNA was measured by a semiquantitative strand-specific reverse transcription-polymerase chain reaction (RT-PCR). Excess liver iron was stained in the liver of 36 patients (30%). Siderotic patients had the same geographic origin, serum and liver HCV RNA levels and H63D and C282Y mutations frequency as non-siderotic patients. However, siderotic patients were older (P = 0.015), more frequently males (P = 0.02), less frequently infected with HCV genotype 3 (P = 0.037) and had a higher liver fibrosis score (P = 0.008). The liver iron content did not correlate with the serum or liver HCV RNA titers. Ten of the 36 patients with liver siderosis had neither a history of excess alcohol intake, multiple transfusions, or HFE mutations. In conclusion, the pathogenesis of the liver iron overload in chronic hepatitis C patients cannot be fully explained by the occurrence of HFE mutations. The exact mechanism of iron accumulation in these patients therefore remains unexplained. ***J. Med. Virol. 60:21–27, 2000.***

KEY WORDS: chronic viral hepatitis; alfa-interferon; viral pathogenesis; iron overload; HFE gene

INTRODUCTION

Chronic hepatitis C is often associated with intrahepatic iron overload [Di Bisceglie et al., 1992; Piperno et al., 1995; Farinati et al., 1995; Kaji et al., 1995; Boucher et al., 1997]. In patients with chronic hepatitis C virus (HCV) infection serum and liver markers of iron overload are elevated more frequently than among patients with chronic liver disease of etiology other than HCV, except for hereditary hemochromatosis [Farinati et al., 1995; Kaji et al., 1995]. This observation has been shown to have clinical significance. Excess iron in the liver may contribute to the necroinflammatory damage, hence accelerating progression towards the development of cirrhosis [Tsukamoto et al., 1995; Beinker et al., 1996; Farinati et al., 1996; Bonkowsky et al., 1997; Mandishona et al., 1998]. Liver iron overload, especially in sinusoids and portal tracts, may also negatively affect the response to α-interferon (α-IFN) treatment [Clemente et al., 1994; Olynyk et al., 1995; Banner et al., 1995; Arber et al., 1995; Izumi et al., 1996; Di Marco et al., 1997; Kaji et al., 1997] even

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though preliminary clinical trials using venesection prior to α-IFN administration have failed so far to show a beneficial effect on the long-term virological response rate [Van Thiel et al., 1996; Fong et al., 1998].

If patients with excessive alcohol intake, repeated blood transfusions, and other causes of excess iron load (such as iatrogenic) are carefully excluded [Shaheen et al., 1998], the hepatic iron content may still be increased in a significant proportion of chronic hepatitis C patients, although only a few patients may have severely increased iron load [Haque et al., 1996]. The pathogenesis of hepatic iron overload in these patients is unknown.

The recent discovery of the putative gene involved in the pathogenesis of hereditary hemochromatosis (HFE) [Feder et al., 1996] and other secondary iron accumulation states [Roberts et al., 1997; Yaouanq et al., 1997; Sampietro et al., 1998; Bonkowsky et al., 1998] has permitted a retrospective review of patients with hepatitis C in order to try to examine correlations between the degree of liver siderosis and the two major HFE gene mutations, i.e. the C→G transition at nucleotide 187 of the open reading frame resulting in an amino acid substitution of histidine by aspartic acid within codon 63 (H63D) and the G→A transition at nucleotide 845 resulting in amino acid substitution of cysteine by tyrosine within codon 282 (C282Y) [Feder et al., 1996].

MATERIALS AND METHODS

Patients

One hundred and twenty chronic hepatitis C patients seen at our institution from November 1994 until March 1998 consented to participate in the study. All had persistent HCV infection, as documented by presence of HCV RNA in serum detected by a 2nd generation qualitative RT-PCR assay (Amplicor™, Roche, Switzerland; sensitivity limit 100 copies/mL) for at least 6 months. There were 80 males and 40 females. The mean age was 42.04 ± 10.38 years (range 19–66). The country of origin was Switzerland in 55 cases, Italy in 20, Spain in 8, other European countries in 13, Africa in 14, Asia in 7 and the Americas in 3. Thirty-seven patients admitted excessive alcohol intake in their history (i.e., they had consumed at least 80 g/d or 40 g/d of alcohol for more than 10 years, males and females, respectively). Two more patients had received repeated blood transfusions (one was undergoing hemodialysis and the other a hematological malignancy). Fifty patients had no identifiable source of HCV infection, 23 had a history of blood transfusion, 42 of intravenous drug addiction, 3 were health-care workers, 1 had been infected via bone marrow transplantation and 1 had multiple tattoos as the only risk factor of HCV.

Forty-nine patients received a course of treatment with α-IFN at a dose of 3 MU, subcutaneously, three times a week. The liver iron status was not considered an eligibility criterion for treatment. Based on the presence or absence of detectable HCV RNA in serum (by the same qualitative RT-PCR assay) after three months of treatment, these patients were considered as virological responders (n = 17) or non-responders (n = 32) to treatment, respectively. Four patients who had a primary virological response and maintained it throughout a six month follow-up period after the end of treatment were considered as sustained responders.

Serum Assays

Serum samples were collected at the time of the liver biopsy and stored at −80° C until use. Apart from routine evaluation of liver function, assays included the measurement of serum ferritin level and of the transferrin saturation, and the detection of HCV RNA by qualitative RT-PCR (Amplicor™, Roche, Switzerland) or by a quantitative signal amplification-based, branched-chain DNA (bDNA) assay (Quantiplex™ version 2, Chiron Corp., Emeryville, CA). HCV RNA genotyping was carried out by restriction fragment length polymorphism of sequences amplified in the 5′ noncoding region of HCV genome [Davidson et al., 1995].

Liver Studies

A liver biopsy was obtained from all patients. In the 49 patients who received α-IFN, the biopsy was undertaken within one year before start of therapy. All liver specimens were divided into two portions. One of these portions was formalin-fixed, paraffin-embedded and evaluated histologically. Histological diagnoses were established according to internationally accepted criteria [Desmet et al., 1994]. H&E-stained sections were evaluated according to a scoring system which includes the semi-quantitative assessment of liver disease grading and staging [Desmet et al., 1994]. Perl's stained slides were also evaluated for the presence and extent of iron deposits by a semi-quantitative score, the histochemical hepatic iron index (HHII) [Deugnier et al., 1993]. This index has been shown to be highly correlated with the biochemical hepatic iron index in patients with genetic HFE [Deugnier et al., 1993]. It also allows definition of the intralobular distribution of iron deposits and to quantify the degree of iron accumulation in hepatocytes versus other, non-parenchymal liver cell populations, which was shown to have clinical significance [Kaji et al., 1995; Bonkowsky et al., 1997]. A correction was applied to the HHII in case of heterogeneous lobular iron distribution [Turlin and Deugnier, 1997].

A specimen adjacent to the one being processed for routine histological studies was snap-frozen in liquid nitrogen and stored at −80° C until use for HCV RNA analysis and HFE mutations analysis.

Total liver RNA was extracted by the acid guanidinium thiocyanate-phenol-chloroform procedure (Tri-Reagent®, Sigma Chemical Co., St. Louis, MO). A strand-specific RT-PCR assay was used to assess the presence and relative titers of either HCV RNA strand, as reported previously [Negro et al., 1998; Negro et al., 1999]. Semi-quantitation was achieved by carrying out a nested RT-PCR to the endpoint on 2- to 4-fold dilutions (in 10 μg/ml of *Escherichia coli* tRNA [Sigma]) of

TABLE I. Characteristics of 120 Chronic Hepatitis C Patients With and Without Stainable Excess Iron in the Liver

	Siderosis (n = 36)	No siderosis (n = 84)	*P*
Age	45.6 ± 9.6	40.5 ± 10.4	0.015
Sex (male to female)	30/6	50/34	0.02
History of alcohol abuse	15	21	NS
Polytransfused	2	0	NS
Geographic origin			
Switzerland	17	38	NS
Italy	5	15	NS
Spain	3	5	NS
Other*	11	26	NS
Source of HCV infection:			
sporadic	17	33	NS
post-transfusion	7	16	NS
intravenous drug addiction	9	33	NS
other	3	2	NS
Serum ferritin (ng/ml)	431 ± 232	169 ± 201	<0.001
Transferrin saturation	0.40 ± 0.19	0.26 ± 0.13	<0.001
Hepatitis grading scores:			
portal/periportal inflammation	2.42 ± 0.79	2.37 ± 0.98	NS
lobular inflammation	1.5 ± 0.67	1.33 ± 0.84	NS
Hepatitis staging score:			
fibrosis	2.06 ± 1.37	1.31 ± 1.39	0.008
HCV RNA levels:			
serum (genomes-Eq × 10^6/ml)	9.39 ± 14.11	10.44 ± 18.33	NS
liver (titer of genomic-strand)	6930 ± 19500	9589 ± 41736	NS
liver (titer of minus-strand)	193 ± 615	56 ± 99	NS
HCV genotype:			
1	20	36	NS
2	5	6	NS
3	3	23	0.037
4	5	9	NS
NA	3	9	NS
mixed (1,2)	0	1	NS

*See Materials and Methods for details.
NA, not assigned; NS, not significant. When applicable, data are presented as mean ± standard deviation.

an initial amount of 100 ng of total liver RNA. Titers were expressed as the last dilution giving a visible band of the appropriate size on a 1.6% agarose gel stained by ethidium bromide. Intrahepatic genomic- and minus-strand HCV RNA titers were normalized to an arbitrary β-actin mRNA titer of 1,024, as measured on the same specimen [Nakajima-Iijima et al., 1985]. The intrahepatic β-actin titer was expressed as the last 4-fold dilution giving a visible band by ethidium bromide staining of RT-PCR products obtained from 100 ng of total liver RNA and run on a 1.6% agarose gel.

HFE Mutations Analysis

Mutational analysis of the HFE gene was carried out on DNA extracted from the liver at the time of the liver biopsy and amplified by polymerase chain reaction (PCR), followed by restriction endonuclease digestion [Feder et al., 1996]. The PCR and digestion products were run onto a 3% agarose gel (Nu-Sieve™, SMC Bioproducts, Rockland, ME) and visualized by ethidium bromide staining. For both H63D and C282Y mutations appropriate positive and negative controls were run in the same enzyme reaction.

Statistical Analysis

Whenever a serum HCV RNA level was below the sensitivity of bDNA assay, a value of 200,000 genomes/mL was arbitrarily used for calculations. Levels of HCV RNA in serum (genomes/ml) and liver (normalized titers) were log-transformed before statistical calculations. Differences among virological and histological variables of different groups of liver biopsy specimens were evaluated by the Student's *t* test (parametric variables) or by the Mann-Whitney's U test (non-parametric variables). The correlations among continuous variables were evaluated by calculating the correlation coefficient; the Spearman's rank correlation test was applied to non-parametric variables. Tables of contingency were evaluated by the χ^2 method or the Fisher's exact test (when $n < 40$).

RESULTS

Clinical and Virological Features of Patients With Liver Siderosis

Thirty-six patients (30%) had a iron accumulation stained histochemically in the liver, with an average HHII of 0.18 ± 0.12 (range 0.008 – 0.36). They tended to be older ($P = 0.015$) and more frequently males ($P = 0.02$) than the remaining 84 patients without histochemical liver siderosis (Table I). Moreover, they had higher serum ferritin levels and higher transferrin saturation rates. There were no differences as to geographic origin and risk factor for HCV infection.

HCV RNA levels in serum and liver (both genomic-

TABLE II. Prevalence of Mutations of the HFE Gene in 120 Chronic Hepatitis C Patients

H63D	C282Y	n (%)
–/–	–/–	79 (65.9)
+/–	–/–	27 (22.5)
+/+	–/–	6 (5)
–/–	+/–	6 (5)
–/–	+/+	1 (0.8)
+/–	+/–	1 (0.8)

and minus-strand) were comparable between the two groups of patients (Table I). No correlation was found either between the HHII and the HCV RNA level in serum ($r = -0.178$; $P = 0.36$) or in liver, either as genomic-strand ($r = -0.255$; $P = 0.44$) or minus-strand titer ($r = 0.3$; $P = 0.36$; Spearman's rank correlation test).

Patients with siderosis were infected less frequently with genotype 3 ($P = 0.037$), whereas the other type distribution (HCV genotypes 1, 2 and 4) was comparable between the two groups of patients (Table I).

Prevalence of HFE Gene Mutations

The overall HFE mutations frequency among the 120 chronic hepatitis C patients was 0.167 for the H63D mutation and 0.033 for the C282Y mutation. Twenty-seven and 6 patients were heterozygotes or homozygotes, respectively, for H63D. Six patients were heterozygotes and one homozygote for C282Y, and 1 patient was a compound heterozygote (Table II).

The 36 patients with liver siderosis had a HFE mutations frequency of 0.194 for H63D and of 0.069 for C282Y (Table III). Among the 84 patients without liver siderosis, the H63D and the C282 mutations had a frequency of 0.155 and 0.024, respectively. These differences were statistically not significant ($P = 0.796$ for H63D and $P = 0.506$ for C282Y).

Among the 36 patients with liver siderosis, 19 had no environmental factors likely to be associated with iron overload (i.e., excess alcohol consumption, repeated blood transfusions or previous exogenous iron use) (nine from group *a*) and all ten from group *b*) as detailed in table 3). The HFE mutation frequency among these 19 patients was 0.26 for the H63D and 0.079 for the C282Y mutation, respectively. Sixty-three patients without siderosis in the liver had none of the above environmental risk factors for iron accumulation (data not shown in detail): the HFE mutation frequency among the latter ones was 0.151 for H63D and 0.032 for C282Y. Although these rates were higher in the former group of patients, the differences were not statistically significant ($P = 0.454$ for H63D and $P = 0.81$ for C282Y).

Overall, 12 of the 36 patients with liver siderosis had at least one environmental factor likely to be associated with iron accumulation, 9 had at least one HFE mutation without other exogenous factors, and 5 had both at least one HFE mutation and an environmental cause of liver siderosis (Table IIIa). As a result, 10 out of 36 siderotic patients (30.6%) had neither HFE mutations nor environmental factors of iron accumulation (Table IIIb). Their average HHII was 0.13 ± 0.07 (range 0.074 –0.31). The HHII calculated for the remaining 26 patients with at least one factor likely to be associated with liver siderosis was 0.2 ± 0.13. The difference did not reach statistical significance ($P = 0.12$).

Histopathological Features of Patients With Liver Siderosis

Patients with siderosis in the liver had comparable levels of portal/periportal or lobular inflammation to that of patients without siderosis (Table I). However, they had significantly higher levels of fibrosis ($P = 0.008$). Among the 36 patients with stainable iron in the liver, the HHII was not correlated with the hepatitis grading scores of the lobular ($r = 0.15$; $P = 0.65$) or the portal/periportal necroinflammation ($r = 0.32$; $P = 0.33$) or with the liver fibrosis score ($r = -0.11$; $P = 0.73$; Spearman's rank correlation test).

Histopathological Grading and Staging and HFE Mutations

The occurrence of a HFE mutation at codons 63 or 282 or both was not correlated with the scores of the lobular ($\chi^2 = 20.629$, $P = 0.193$) or of the portal/periportal activity ($\chi^2 = 18.066$; $P = 0.32$) or of the fibrosis ($\chi^2 = 17.319$, $P = 0.632$).

Intrahepatic Distribution of the Iron Deposits

Overall, 27 out of 36 patients (75%) had iron deposits predominantly (19 cases) or exclusively (8 cases) in the hepatocytes. Seven patients had an iron accumulation exclusively confined to Kupffer's cells (Table III). In the remaining 2 patients (both had multiple transfusions as a risk factor for iron overload), the score of the iron staining was comparable for hepatocytes and sinusoidal cells. Among the 10 patients without excess alcohol intake, repeated transfusions or HFE mutations, 8 had predominantly (4 patients) or exclusively (4 patients) iron deposits within hepatocytes, while the remaining 2 patients had exclusively iron accumulation in Kupffer's cells (Table III). A low-level portal iron accumulation was observed in 5 patients and was confined to rare macrophages in the portal tract. No iron deposits were seen in biliary tract or vascular endothelium cells.

Presence of Liver Siderosis and Response to α-IFN Treatment

Forty-nine patients received an α-IFN treatment at the dose of 3 MU, subcutaneously, three times a week, for at least three months. Two out of 12 siderotic patients responded to treatment, as compared to 10 out of 37 non-siderotic patients. This difference was statistically not significant ($\chi^2 = 1.348$, $P = 0.246$).

DISCUSSION

It was found that iron accumulates in the liver of about one-third of chronic hepatitis C patients, and

TABLE III. Characteristics of 36 Chronic Hepatitis C Patients With Liver Siderosis

a) Patients with at least one risk factor (genetic and/or environmental) for liver siderosis

#	Age (y)	Sex	Deugnier's score				H63D	C282Y	Staging score*	Environmental risk factors
			Hep	Sin	Port	HHII				
1	43	f	0	1	0	0.008	–/–	–/–	0	Alcohol
2	43	m	0	2	0	0.046	–/–	–/–	2	Alcohol
3	44	m	3	0	0	0.067	–/+	–/–	0	None
4	42	f	3	3	0	0.071	–/–	–/–	2	Transfusions
5	42	m	0	4	0	0.093	+/+	–/–	0	None
6	62	m	6	1	0	0.111	–/–	–/–	4	Alcohol
7	53	m	6	0	0	0.111	–/–	–/–	2	Alcohol
8	61	m	6	0	0	0.113	–/–	–/–	3	Alcohol
9	59	f	6	1	0	0.117	–/+	–/+	1	None
10	47	m	18	0	0	0.125	–/–	–/–	4	Alcohol
11	33	m	0	4	0	0.15	–/–	–/–	4	Alcohol
12	40	m	0	6	0	0.15	–/–	–/–	2	Alcohol
13	42	m	6	1	0	0.163	–/–	–/–	4	Alcohol
14	34	m	6	0	0	0.17	–/+	–/–	2	None
15	38	m	6	0	1	0.171	+/+	–/–	1	None
16	43	m	6	2	0	0.182	–/–	–/–	4	Alcohol
17	32	m	6	0	0	0.182	–/+	–/–	1	None
18	51	f	6	2	0	0.19	–/+	–/–	1	None
19	65	m	15	0	2	0.262	–/+	–/–	4	None
20	41	m	6	6	0	0.28	–/+	–/–	4	Alcohol
21	33	m	6	4	0	0.29	–/–	–/–	0	Alcohol
22	38	m	9	2	0	0.29	+/+	–/–	2	Alcohol
23	28	m	15	2	0	0.36	–/–	–/+	2	Alcohol
24	49	m	18	6	0	0.48	–/–	–/+	4	Alcohol
25	53	f	18	7	1	0.48	–/+	–/–	1	Transfusions
26	44	m	21	0	1	0.489	–/–	+/+	1	None

b) Patients without environmental or genetic risk factors for liver siderosis

#	Age (y)	Sex	Deugnier's score				Staging score*
			Hep	Sin	Port	HHII	
27	47	f	3	0	0	0.064	3
28	53	m	0	4	0	0.074	2
29	47	m	0	4	0	0.083	2
30	44	m	3	2	0	0.11	0
31	53	m	3	2	1	0.11	2
32	63	m	6	2	0	0.12	3
33	46	m	6	0	0	0.13	2
34	57	m	6	2	0	0.14	3
35	41	m	6	0	0	0.143	0
36	29	m	9	0	0	0.31	2

*Staging score: score of the liver fibrosis, as defined in Desmet et al. [1994].
y, years; f, female; m, male; Hep, score of iron deposits in hepatocytes; Sin, score of iron deposits in sinusoidal cells; Port, score of iron deposits in portal cells; HHII, histochemical hepatic iron index corrected for heterogeneous lobular iron distribution (see Materials and Methods for details).

that in almost one-third of these patients there are no HFE mutations or exogenous factors accounting for the iron overload. To make this statement more stringent, it was assumed that even the H63D mutation in its heterozygous form may be associated with iron overload, although the clinical significance of this mutation probably need not be emphasized [Feder et al., 1996]. Moreover, although some patients with the C282Y mutation and/or heavy alcohol intake had the highest degrees of iron overload, the average HHII was the same in patients with environmental factors or siderosis and/or HFE mutations as compared to patients without. Recent reports from other groups [Hezode et al., 1998; Kazemi-Shirazi et al., 1999], who showed that HFE mutations cannot entirely explain the liver siderosis of patients with chronic hepatitis C without other risk factors for iron overload are confirmed by the present study.

The frequent occurrence of iron accumulation in the liver of chronic hepatitis C patients has been recognized for some time. With the exception of the genetic HFE, liver iron overload seems to be found more often in chronic hepatitis C than in other chronic liver disease states [Di Bisceglie et al., 1992; Piperno et al., 1995; Farinati et al., 1995; Kaji et al., 1995; Boucher et al., 1997]. Although the degree of iron accumulation may be less than in the case of genetically determined or alcohol-associated liver disease, it may nonetheless bring about important clinical consequences such as an accelerated progression towards cirrhosis and liver cancer [Tsukamoto et al., 1995; Beinker et al., 1996; Farinati et al., 1996; Bonkowsky et al., 1997; Mandis-

hona et al., 1998; Piperno et al., 1998] and a lower rate or response to α-IFN treatment [Clemente et al., 1994; Olynyk et al., 1995; Banner et al., 1995; Arber et al., 1995; Izumi et al., 1996; Di Marco et al., 1997; Kaji et al., 1997]. A better understanding of the mechanisms underlying the iron accumulation may therefore improve the management of these patients.

According to an hypothesis, the iron may accumulate due to the continuous release from damaged hepatocytes, either through a direct cytopathic effect of the virus or *via* the immune-mediated cell lysis [Bonkowsky et al., 1997]. Although the pathogenesis of chronic hepatitis C is believed to be mostly due to the host immune response [Ballardini et al., 1995] rather than to a cytopathic effect of the virus, we nonetheless looked for a relationship between HCV replicative levels (in serum and/or liver) and degree of siderosis, but found no such correlation. The amount of siderosis was not correlated with the degree of necro-inflammatory activity either, although this may vary over time. A better indication of a relationship between ongoing liver damage and iron accumulation may come from the fibrosis score. This was found to be higher among patients with siderosis than among patients without, even though the two scores did not correlate with each other. However, the continuous immune lysis of liver cells would result in a preferential accumulation of iron in Kupffer's cells, as reported by some investigators [Haque et al., 1996; Boucher et al., 1997]. In contrast, the majority of patients with siderosis in the liver considered in the present series had iron deposits predominantly or exclusively in hepatocytes, and this also held true among the ten patients who had neither HFE gene mutations nor other evident factors of iron overload.

Alternatively, the iron accumulation occurring in chronic hepatitis may be explained by an increased intestinal iron absorption [Chapman et al., 1982; LeSage et al., 1983] or by an ineffective erythropoiesis [Uchida, 1995]. The latter hypothesis is intriguing in the case of chronic hepatitis C in view of the reported localization of HCV in cells of the hematopoietic lineage [Bronowicky et al., 1998; Lérat et al., 1998]. It is noteworthy that HFE mutations have not been found to be associated with the iron overload in sideroblastic anemia patients [Beris et al., 1999]. Finally, iron accumulation may be mediated by some interleukins, such as interleukin-1 [Dinarello, 1988] or interleukin-11 [Baynes et al., 1995], or by some macrophage-derived iron-regulating factors, as already described in other viral infections [Mathur et al., 1990].

Whatever the pathogenesis of iron accumulation in chronic hepatitis C patients, this is likely to correlate with the duration of the liver disease. Although we could not ascertain the date of infection with HCV for most of the patients, there is some indirect evidence to support this hypothesis. In fact, patients with liver siderosis tended to be older, to have a higher degree of liver fibrosis, and to be infected more rarely with HCV genotype 3, which is of more recent introduction and spread among HCV-positive population in the Western countries [Sacco et al., 1997]. Again, the occurrence of HFE mutations did not appear to correlate with the score of fibrosis. In particular, the C282Y heterozygous state was not associated with a higher degree of fibrosis, in contrast with data by Smith et al. [1998], but in agreement with a more recent report [Hezode et al., 1999].

Iron overload may also negatively affect the response to α-IFN treatment. We could not confirm this general view, but our negative observation was most likely due to the smaller number of patients who were treated.

In conclusion, it was shown that as many as one-third of patients with chronic hepatitis C and siderosis of the liver have neither environmental factors nor major HFE gene mutations which could account for the iron accumulation. Other causes may play a role in the iron overload of hepatocytes: these may encompass hitherto unknown HFE (or other genes) mutations (especially in patients of non-European origin), viral genetic microheterogeneity or other host- or viral-mediated factors. Further studies are warranted to determine the pathogenesis of iron accumulation in the liver of chronic hepatitis C patients.

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